ANNUAL REPRODUCTIVE CYCLES OF THE CHITONS, KATHERINA TUNICATA AND MOPALIA HINDSII [1]

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The reproductive season of marine invertebrates has been determined in a variety of ways: spawning, the appearance of young in plankton, increase in size of gonad relative to the body, and development of ripe gametes (see Giese, 1959, for reference). In most cases an annual reproductive season has been observed, but many species breed more than once during the season, some showing a monthly rhythm (Korringa, 1947). Data on reproductive seasons of marine invertebrates are still not extensive and the problems posed are numerous. It is desirable that data on many more species be gathered to gain a better perspective. This paper records observations on reproduction in two species of chitons, *Katherina tunicata,* an intertidal form, and *Mopalia hindsii,* a pile dweller.

Methods

Ten specimens of *Katherina* were gathered monthly from the mid-littoral zone of Carmel Point during 1954–55 and from the same zone at Yankee Point during 1956–58, in each case from rocks in the *Postelsia* and mussel zone exposed at low tide. The specimens at Carmel Point were larger but the population was sparser, and perhaps less representative than that at Yankee Point. The sample from Yankee Point was therefore selective inasmuch as very small specimens were avoided (weights: 1957, 16–44 gms.; 1958, 11–53 gms.)

Ten specimens of *Mopalia* were obtained during monthly low tides from the pilings at Monterey harbor. The population in this region is unusual in that the specimens are preponderantly large and not too numerous, therefore the samples may not be truly representative of the species (weights: 1957, 14–73 gms.; 1958, 16–65 gms.). However, this species was not found in other accessible environments in sufficient numbers for sampling.

Each chiton was weighed, the foot removed and the animal eviscerated, permitting easy removal of the gonad. The gonad volume was determined and the gonad index (the volume of the gonad is divided by the weight of the animal and multiplied by 100) determined.

To determine whether any striking changes in the content of nutrients occurred in the blood during the course of the reproductive cycle of *Katherina,* the reducing sugar, protein and non-protein nitrogen were determined monthly in samples of blood. For this purpose the blood of from ten to twenty individuals

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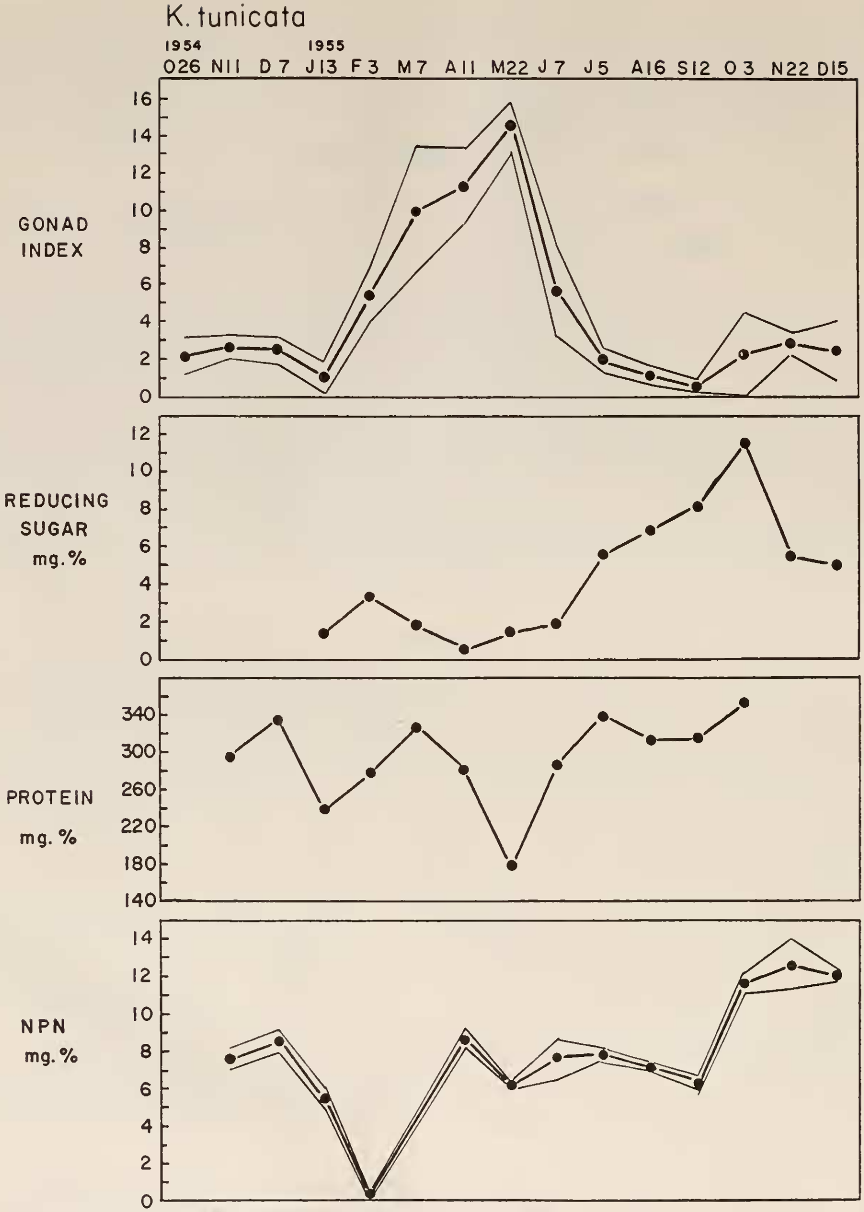

FIGURE 1. Top: Reproductive cycle of *Katherina tunicata* for 1955 as determined by the gonad index. The gonad index is the ratio of gonad volume to wet weight of the entire animal times 100. The specimens were collected at Carmel Point, California. The dots indicate the means and the lines, the 95 per cent confidence limits. Lower three graphs show variation in reducing sugar, protein and non-protein nitrogen in the population samples used for gonad analyses.

had to be pooled because a single animal does not have enough for analysis, a 33-gram animal giving only several milliliters of blood. Blood samples were obtained from the lateral sinuses following puncture through the mantle cavity on either side of the animal. The blood is a yellowish fluid which was not observed to coagulate although it showed some murkiness on standing, suggesting a feeble clumping of the blood corpuscles.

Reducing sugar was determined by the anthrone method (Seifter *et al.,* 1950) after precipitating the proteins with 10 per cent trichloracetic acid. Non-protein nitrogen was determined with the micro-Kjeldahl after precipitating the protein,

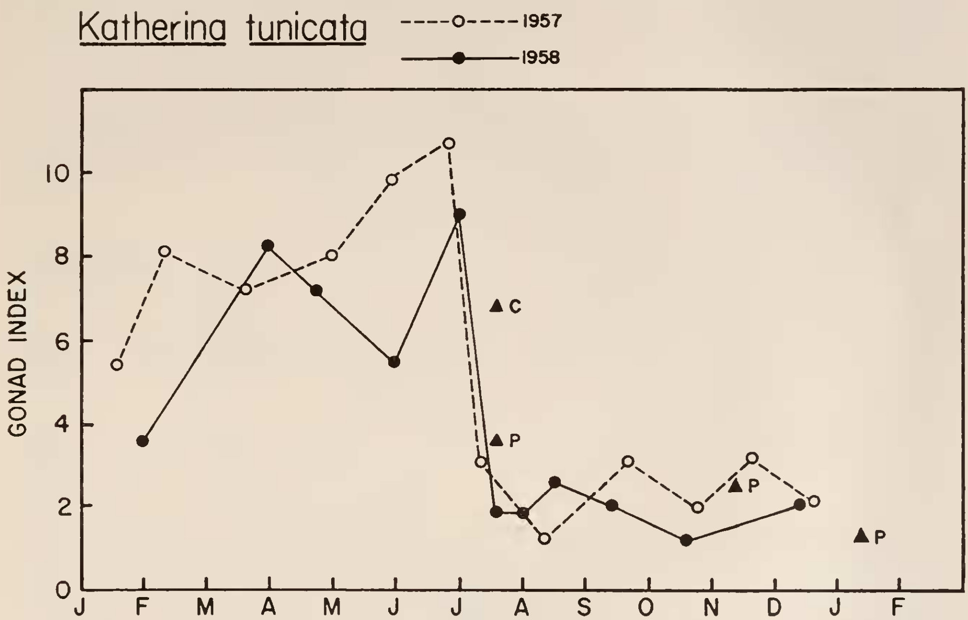

FIGURE 2. Reproductive cycle for *Katherina tunicata* during 1957 and 1958 as determined by gonad index. The specimens were collected at Yankee Point, with the exception of points marked C which came from Carmel Point and those marked P which came from Pescadero Point.

and total nitrogen was determined by the same methods, without the preliminary addition of protein-precipitating agents. Protein was taken to be the difference between these two volumes multiplied by 6.25.

KATHERINA TUNICATA

For each of the three years during which *Katherina* was studied an annual reproductive cycle was found. It is most clear-cut for the year 1954-55 (the population collected at Carmel Point) as shown in Figure 1. It is less distinct for the collections at Yankee Point during the years 1956–57 and 1957–58, the gonad index being high for several months preceding the spawnout, as shown in Figure 2. The difference in sharpness of the cycle at the two locations may be the result of a more homogeneous population of large animals at Carmel Point. A comparison of the gonadal index for random specimens from both localities during the same year is desirable.

During the height of the season males are easily distinguished from females by the color of the gonad, which is greenish in the female (the eggs being green) and very light in the male (the sperm being white). However, it is not possible to distinguish the sexes of individuals from spawned out gonads, but the shell gland of the female is always diagnostic of sex even in a spent female. When minute spherical eggs begin to develop as a new cycle comes into prospect, diagnosis from gonads is again easy. Statistical analysis of the 1954–55 data showed no essential difference in the time of onset in maturity of testes and ovaries in males and females; therefore the data for both sexes were combined. It was, however, noted that the gonad index of males appears to be slightly higher than that of females at the height of the season and lower than that of the females during the decline of the season. The 95 per cent confidence limits for the pooled males and females given in Figure 1 indicate that the entire population of animals is relatively homogeneous, since the confidence band is never very wide.

The data for almost two years of the breeding cycle for the same population at Yankee Point, given in Figure 2, indicate that the cycle varies somewhat from year to year. After slowly spawning during the months of March, April, May and June of 1958 the gonad index again rose, suggesting minor ripples in sexual activity or a second active period because of favorable conditions in the area. During this second cycle the gonad index rose significantly from 5.5 on June 9 to 8.9 on July 9 then fell precipitously to 1.9 on July 18 of 1958.

It is interesting to note that the populations sampled at three stations on July 17 and 18 showed different average gonad indices: 6.8 at Carmel Beach, 3.6 for Pescadero Point, and 1.9 for Yankee Point. This indicates that the exact time of spawning is probably in part determined by the geographical and ecological location.

Two findings are similar for the three years of data available; 1) in all cases the chitons have small shrunken gonads late in July, and 2) the gonads remain shrunken during the late summer, fall and early winter. In the months after the beginning of winter the gonads grow rapidly and gametogenesis occurs. The reproductive cycle therefore consists of several months of stasis, several months of growth, several months during which the gonads are ready for spawning, and probably several periods of spawning. MacGinitie and MacGinitie (1949) report that *Katherina* lays eggs in the Monterey Bay area in July while Hewatt (1938) reports it spawning in the same area in May, and Rickets and Calvin (1948) say it spawns in Puget Sound a month or two earlier.

Attempts to study breeding and spawning of *Katherina* in the laboratory did not prove successful since often the animals crawled out of the tanks to dry locations, and even though periodically submersed, they deteriorated. They did not seem to eat while in the laboratory although red and brown algae were provided and a scum of diatoms was always present on the surface of the aquaria.

In its natural environment *Katherina* feeds upon diatoms and upon algae, since the pellets in the gut of animals sampled consisted of partially digested algae of various kinds, including brown and red algae. In other cases the gut was filled from end to end with diatoms and skeletons of diatoms. Apparently the chitons eat what is readily available to them and are always found within reach of algae. *Katherina* does not move out of the sunlight, remaining on the upper surfaces of the rocks during low tide. It is therefore subjected to and weathers all the changes in conditions which obtain during low tide.[3] Most chitons have tegmental aesthetes or "shell eyes" which enable them to perceive the light. These are absent in *Cryptochiton* and may be of less importance in *Katherina* in which much of the shell is covered by tissue (Crozier, 1921). A variety of other organisms are found growing upon *Katherina*. These include occasional barnacles, coralline algae, and colonial animals such as bryozoans and hydroids, all of which anchor to the skeletal plates. The overgrowth by algae and various colonial animals indicates the sessile nature of the animals and their tendency to remain on the upper surface of the rocks.

The nutrients in the blood of *Katherina* vary during the year (Fig. 1). The most prominent constituent of the blood is protein which is always present in large amounts, varying from about 180 to 240 mg. per cent. The lowest values of blood protein appear to coincide with the highest gonad index. The non-protein nitrogen of the blood is small in amount, reaching a maximal value of about 12 mg. per cent and falling to almost zero when the gonad is increasing most rapidly in size. Reducing sugar is present in rather small amounts from almost zero to about 11 mg. per cent. A single determination of the blood constituents therefore has little meaning as a value for the species since the constituents vary so much in amount from month to month. The variations in blood constituents in Figure 1 may possibly be correlated with the breeding season. Similar variations in content of organic constituents of the body fluid of echinoderms (Bennett and Giese, 1955), and in the blood of a number of species of crabs (Leone, 1953) have also been documented. It would be interesting to know whether the blood of marine invertebrates is generally so variable.

A certain amount of organic material is stored in the foot, the gonads and the hepatic gland. It is clear from preliminary tests that neither the digestive gland nor the foot has much glycogen, although almost 0.1 per cent wet weight of the soft tissue of the animal consisted of glycogen (0.83 per cent of the dry weight). Lipid seems to be more important than glycogen as a storage material. Detailed studies on these constituents are under way.

Mopalia hindsii

Mopalia hindsii is listed by MacGinitie and MacGinitie (1949) as an estuarine form which is often found on pilings. They have found some specimens as large as 10 centimeters long and 7.5 centimeters wide. The animals used in this study were usually smaller than the maximum, being generally about 8.0 centimeters long and 5.5 centimeters wide. Specimens from the pilings in Monterey Harbor were covered by a forest of bryozoans, hydroids, and a multitude of larval crustaceans,

[3] Heath (1899, 1905) found that many of the chitons are highly sensitive to sunlight and creep into crevices, and that one species, *Ischnochiton magdalenensis*, even buries itself in the sand with the approach of day. *Katherina tunicata* and *Cryptochiton stelleri* are among the few species which remain exposed during the bright daylight. *Mopalia hindsii*, it is true, remains on the inner pilings at Monterey Harbor during the day, but this is an environment of rather attenuated light.

nemerteans, roundworms, annelid worms, and protozoans (including the large ciliate *Condylostoma*). This association is common to this species (MacGinitie and MacGinitie, 1949). Shells of *Mopalia* are often weakened by a boring worm (Tucker and Giese, 1959), probably of the family Spionidae.

The intestinal contents of the specimens studied were filled with skeletons of bryozoans and other organisms which grow on the pilings which they inhabit. *Mopalia* was generally absent from the outer pilings where the light is sufficient for a relatively abundant growth of algae and were more numerous in the darker regions where bryozoans, hydroids, anemones, and starfishes occurred.

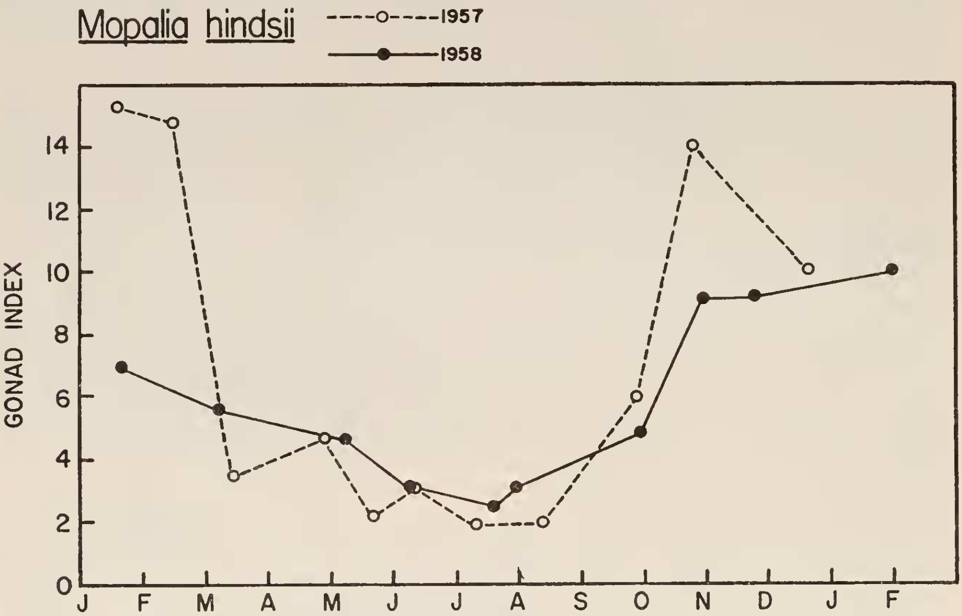

FIGURE 3. Reproductive cycle of *Mopalia hindsii* during 1957 and 1958 as determined by gonad index. Specimens collected on pilings in Monterey Harbor.

A definite annual reproductive cycle is observed for *Mopalia* (Fig. 3). Breeding, as indicated by enlargement of gonads with development of eggs and sperm, occurs in the late fall and winter, approximately from October to February or March. That it was somewhat different each year is indicated by the data in Figure 3.

DISCUSSION

A number of previous workers have described the breeding habits of various chitons (Heath, 1899, 1905; Grave, 1922; Stephenson, 1934; Costello *et al.*, 1957). Grave (1922) found that *Chaetopleura apiculata* spawns at ten-day intervals during the months of June, July and August at Woods Hole, the activity declining in the intermediate periods (for other references, see Costello *et al.*, 1957). Spawning in this species occurred during the night and is possibly associated with phases of the moon (Korringa, 1947). Stephenson (1934) found that *Acanthozostera gemmata* bred in the Great Barrier Reef of Australia from September to April, spawning occurring every four weeks during the breeding period, associated with phases of the moon. After April it entered a prolonged resting period.

Heath (1899, 1905) made many excellent observations of the breeding activities of chitons in nature. He observed *I. magdalenensis* breeding in the Monterey area in May and June, in the latter month over a considerable length of the coastline in the area. Hewatt (1938) also observed the same species breeding in June, but found *Mopalia muscosa* breeding in September. Heath (1899, 1905) found that chitons of the Monterey area do not breed in captivity.

Heath (1905) also observed that isolated males of *I. magdalenensis* and *Mopalia lignosa* spawn, but isolated females do not. However, females placed with males in a tide pool spawn soon after the males have spawned, suggesting chemical stimulation. This observation confirms similar observations by Metcalf (1892) on *Chiton squamosus* and *C. marmoratus* (see also Crozier, 1922). Heath observed shedding and egg laying in *Ischnochiton mertensii, I. cooperi, M. muscosa* and *K. tunicata* in large pools isolated by low tides. The males began shedding when the waters became tranquil after recession of the tide and stopped shedding when disturbed by water movements. Shedding sometimes continued for as long as two hours, steadily or in spurts. He bred some chitons in isolated pools and grew them to maturity. Young *K. tunicata* isolated in pools near mean tide mark grew to 25 mm. in length in one year and reached their average length of 55 mm. in three, a rapid rate of growth characteristic of other species of chitons as well.

The two species of chitons discussed in this paper, *K. tunicata* and *M. hindsii,* show a distinct breeding season which, while similar from year to year, is sufficiently distinctive each year to indicate that it is not closely tied to day-length or some other factor invariant from year to year, but rather is subject to action of various local factors. The local variation in reproductive state found in one collection at three different stations in the Monterey area (Yankee Point, Pescadero Point, and Carmel Point) during early July, 1958 indicates how important to the breeding cycle are the small differences in the ecology of the three environments.

The most curious feature of breeding in the two species of chitons studied here is the reciprocal nature of their breeding seasons, *Katherina* breeding in the summer, *Mopalia* in the winter. This resembles the pattern described for the crab *Pachygrapsus crassipes* which breeds in the summer, while *Hemigrapsus nudus,* another grapsoid crab of similar habits and nature, breeds in winter (Boolootian *et al.,* 1959). It would be valuable to ascertain what factors trigger the sweep of the reproductive cycles at a period approximately six months apart in the two species of chitons. Other cases of this type are known but in no case has an adequate experimental analysis of causal mechanism yet been made (Giese, 1959).

Summary

1. The reproductive cycles of two species of chitons, *Katherina tunicata* and *Mopalia hindsii,* collected in Monterey Bay are recorded, the first for three years and the second for two.

2. *Katherina* breeds in the summer, *Mopalia* in the fall and winter.

3. In each case the gonad index (ratio of gonad volume to body weight times 100) rises gradually and falls rather precipitously as spawning occurs. Differences in onset of breeding occurred during the years for which records are available, suggesting timing of events by local conditions.

4. Blood protein, non-protein nitrogen, and reducing sugar vary during the year but it is not clear whether these variations are significantly correlated with reproductive condition.

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